Microreplicas as a Technique for Rapid Evaluation of Surface Silica Micromorphology in Equisetum

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The genus *Equisetum* has caused problems to field botanists and herbarium workers because the species are often difficult to identify. The general morphology is so plastic and responsive to environmental influence that the same species can look quite different under different circumstances, and two different species can under certain conditions closely resemble one another. One source of characters which has recently come to the foreground is the micromorphology of surface silica.

Equisetum has long been noted for its ability to take up silicon dioxide and deposit it on the surface of the plant as a hard, outer coat. For this reason, certain species have been used to scour pots and pans (scouring rush), or to polish wood (joiner's rush). The silica deposits have been suspected of varying among species, and in studying subgenus *Hippochaete*, I used the silica profile of the branch ridges as a character in distinguishing *E. giganteum*, *E. myriochaetum*, and their hybrid, *E.* × *schaffneri* (Hauke, 1963). I also illustrated the silica rosettes in the valleys of *E. ramosissimum* subsp. *debile*. Milde (1867) had illustrated these, as well as some vague differences of surface pattern among species of subg. *Equisetum*, particularly on the stomata.

With development of the Scanning Electron Microscope (SEM), surface features of biological entities have become more amenable to study. Various people have used this technique to look at *Equisetum* (Laroche, 1968, 1969a, 1969b; Laroche et al, 1970; Kaufman et al., 1971; Page, 1972, 1974; Tanowitz, 1975; Dayanandan, 1977; Lawry, unpublished). Page (1972) revised the taxonomy of *Equisetum* subg. *Equisetum* largely utilizing silica micromorphology. Since the SEM technique is somewhat laborious and requires special equipment, it does not lend itself to convenient and rapid evaluation of surface micromorphology of a number of specimens from different localities, of plants of different ages, or of different parts of the same plant. Apparently Page looked at only one specimen for each species, and did not appreciate the possibility of silica pattern variation within a plant or between plants of the same species.

The silica micromorphology does show some consistent characters by which species of *Equisetum* subg. *Equisetum* can be recognized (Hauke, 1978), and it is useful in helping to identify aberrant specimens. However, it is impractical for the working taxonomist, who must sort stacks of specimens, to stop and prepare for the SEM material from a problematic specimen, assuming he even has ready access to a scanning electron microscope.

I sought a practical alternative to the SEM for rapidly evaluating surface silica micromorphology of herbarium specimens of *Equisetum*. The technique I adopted is the "microreplica" method, as published in the Turtox News some years ago. I have used this successfully on dried, pressed herbarium specimens. It works best

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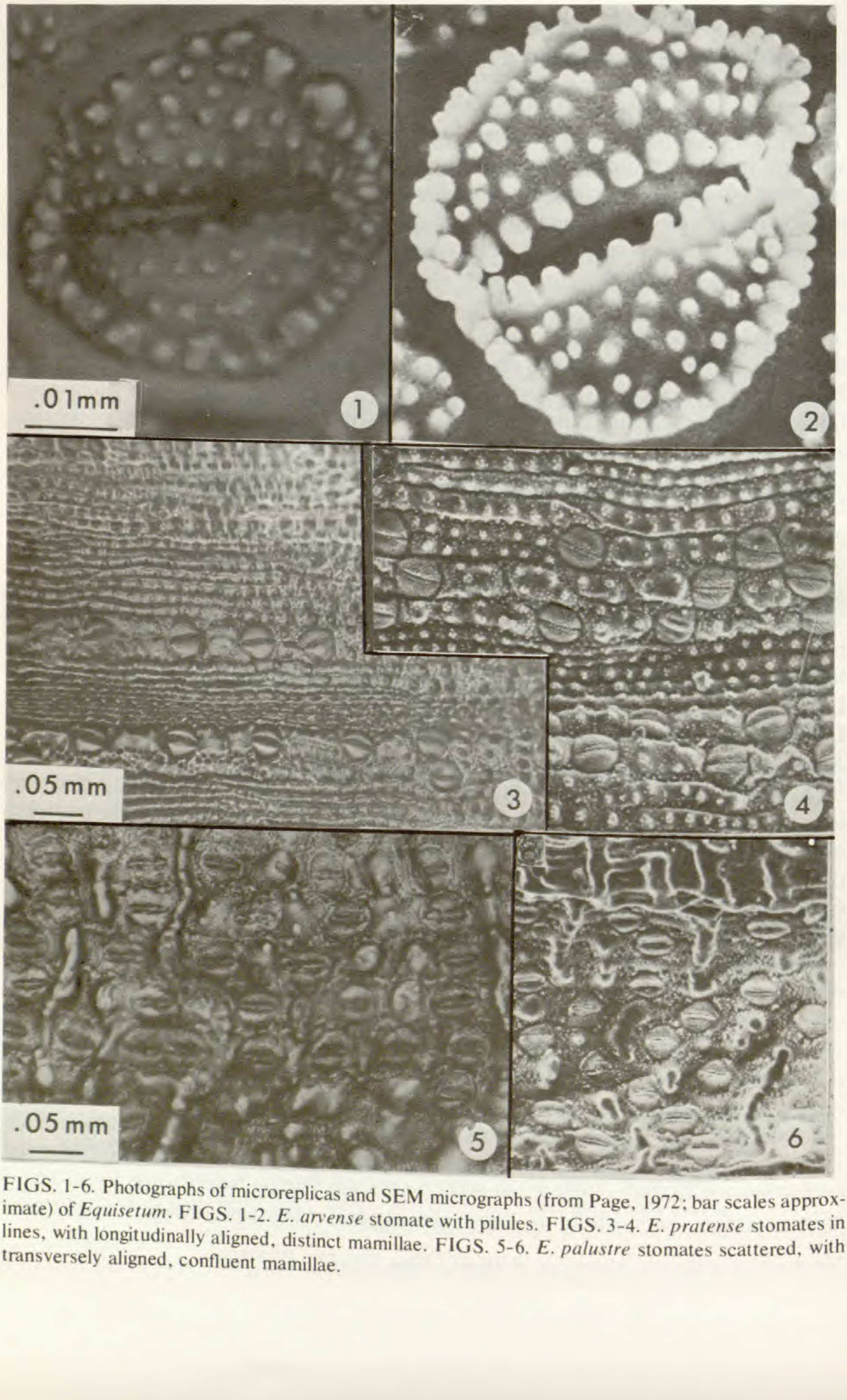

FIGS. 1-6. Photographs of microreplicas and SEM micrographs (from Page, 1972; bar scales approximate) of *Equisetum*. FIGS. 1-2. *E. arvense* stomate with pilules. FIGS. 3-4. *E. pratense* stomates in lines, with longitudinally aligned, distinct mamillae. FIGS. 5-6. *E. palustre* stomates scattered, with transversely aligned, confluent mamillae.

on branches that have been flattened well and are lying against the herbarium sheet.

The portion of branch selected is wetted with acetone, and then a plastic cover slip (22 mm^2) is pressed firmly against it. One can apply maximum pressure by using the end of the thumb, pressed vertically with the weight of the body. After about ½ minute, the coverslip is removed. It is placed on a microscope slide impression side down and observed under a standard light microscope. The acetone will have softened the plastic and the pressure will have caused it to conform to the surface of the branch. Microscopic physical features of the surface will be impressed into the plastic, hence the name "microreplica."

As with any technique, certain precautions are necessary for it to work well. If too much acetone is used, some will move over the coverslip and the technician's fingerprint will be impressed into the plastic. If the surface is not reasonably flattened, only the high spots will be replicated. If there are abrupt heights and depths, the plastic will develop fine fracture lines that will obscure any replica. Care must be used in removing the coverslip from the branch, to avoid having the branch break and its surface stick to the plastic coverslip. I have found that a dissecting needle slid between the two helps to separate them. The coverslip, as it dries, often tends to bend. I immediately attach it to a microscope slide with permanent transparent mending tape to minimize this distortion.

The microreplicas produced by this technique show enough micromorphology to reveal the patterns detected with the SEM. It is true that the contrast is not so great and the resolution of finer details is often poor, but one can see the size, arrangement, and distinctness of mamillae and the type and distribution of pilules on the stomata. *Figure 1* is a photograph of a microreplica showing the stomate and pilules of *Equisetum arvense*, and *Fig. 2* is an SEM micrograph from Page (1972) of the same species. *Figures 3* and *5* are photographs of microreplicas showing the mamillae and stomatal arrangement of *E. pratense* and *E. palustre*, respectively, and *Figs. 4* and *6* are SEM micrographs from Page (1972) of the same species.

As can be seen, there are micromorphological characters useful in species identification, and these characters can be detected quickly on questionable herbarium specimens using the microreplica technique.

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